Case Report

Nell1 as Target Antigen for Mercury Related Membranous Nephropathy: A Case Report

Abstract

Membranous nephropathy constitutes 30% of adult nephrotic syndrome. Of all cases of membranous nephropathy, primary membranous nephropathy is commonest. Mercury is known to be a cause of secondary membranous nephropathy. There is no human data on the pathophysiology of mercury-related membranous nephropathy, but animal studies suggested an autoimmune mechanism behind it. There is no data to the best of our knowledge about target antigen for mercury-related membranous nephropathy. We are reporting a case of NELL-1 positive mercury-related membranous nephropathy that started resolving after stopping siddha medication and taking antiproteinuric. There was also concomitant euthyroid lymphocytic thyroiditis with anti-TPO positive, which started after exposure to siddha medication, which suggests systemic autoimmune phenomenon due to mercury exposure.

Keywords: *Membranous nephropathy, mercury, NELL-1, secondary membranous*

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Background

Membranous nephropathy (MN) constitutes 30% of cases of nephrotic syndrome in adults.[1] Primary membranous nephropathy (PMN) and secondary membranous nephropathy (SMN) constitute 75%–80% of cases and 20%–25% of cases, respectively.[1] Various secondary causes of MN include infections such as HBV, HCV, and HIV; malignancy; autoimmune disorders such as systemic lupus erythematosus; drugs such as gold, penicillamine, and NSAIDs; and toxins such as mercury and lead. Various target antigens are found to be responsible for MN, such as phospholipase A2 receptor (PLA2R), thrombospondin (THSD7A), neural epidermal-like growth factor 1 (NELL1), semaphorin-3b, exostosin-1, and exostosin 2.[1]

Mercury exposure has been seen to cause MN.[2] The exact pathogenesis of mercury-related MN is not known. Animal studies have shown that in mercury-related glomerulonephritis, T-cell-mediated polyclonal B-cell activation leads to various autoantibody production, which is responsible for glomerular pathology.[3] This is the first report to the best of our knowledge mentioning specific target antigens involved in mercury-related MN in humans.

Clinical presentation

A 47-year-old female had menstrual irregularities for 1-year duration for which she took siddha medication (detailed information not available) for 2 months, 4 months back. She presented with both lower limb swelling and facial puffiness for 4 months. On evaluation she had: urine routine- 4+ albumin, 10/high power field (HPF) WBC, 4/HPF RBC; serum creatinine- 0.4; serum albumin- 2.1 gm/dl; total cholesterol- 429 mg/dl, serum LDL- 342 mg/dl, serum HDL- 58 mg/dl, serum triglyceride- 238 mg/dl, and 24-h urine protein- 10 gm, suggestive of nephrotic syndrome. For the last 2 months, she was having swelling in the anterior part of the neck. For evaluation of neck swelling, she underwent fine-needle aspiration as advised by the surgeon and it was suggestive of lymphocytic thyroiditis, Anti-TPO antibody- 384 IU/ml (normal: <50 IU/ml), serum TSH- 2.6 IU/ml.

In view of nephrotic syndrome, she underwent renal biopsy which showed; Light microscopy (LM) did not reveal any significant abnormality in the glomerulus and tubulointerstitium,

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other than mild stiffening of glomerular capillaries, Immunofluorescence (IF): IgG 3+; C3 1+; C1q 1+; Kappa 3+; Lambda 3+ (all capillary wall granular), EM showed scattered subepithelial electron-dense deposits with diffuse effacement of visceral epithelial cell foot processes, consistent with a stage 1 MN [Figure 1].

To evaluate the cause of MN, immunohistochemistry on paraffin block of renal tissue was done for phospholipase A2 receptor (PLA2R), thrombospondin (THSD7A), neural epidermal like growth factor like 1 (NELL1), semaphorin-3b, exostosin-1, and exostosin 2, which showed diffuse NELL1 positivity (2+/3+) along the glomerular capillaries [Figure 2]. Other immunostains were negative. Evaluation for secondary causes of MN revealed elevated mercury in urine- 17.7 microgram/liter (normal: <10 microgram/liter). There was no evidence of connective tissue disorder contributing to MN (ANA negative, normal serum complements, anti-PLA2R antibody negative). There was no evidence of infection contributing to the above (HIV serology, HBsAg negative, HCV RNA negative, and Chest X-ray -no abnormality). In malignancy screening, CECT abdomen, chest X-ray, and mammography showed no evidence of malignancy; PAP smear-no evidence of malignant or premalignant features; stool for occult blood absent; CEA- 3.2 ng/ml (normal: 3.8–5 ng/ml); CA19-9- 2 U/ml (normal: 0–37 U/ml); CA-125- 10 U/ml (normal: <46 U/ml); and AFP- 1.9 ng/ml (normal: <7 ng/ml). She discontinued siddha medication and was started on angiotensin receptor blockers, and 2 months following that, her serum albumin increased to 3.4 gm/dl and proteinuria reduced to 2 gm/24 h (attained partial remission), and was thus continued on antiproteinuric therapy and no chelation was given in view of resolving disease and urinary mercury less than 100 Mcg/L and normal serum mercury.

Discussion

Membranous nephropathy is a common cause of nephrotic syndrome in adults. Heavy metals such as mercury exposure can cause MN like in our case.[2] Mercury-related MN has been seen in patients on indigenous (e.g., siddha) medication [Table 1].[2,4,5] Besides renal involvement mercury-related autoimmune thyroiditis is also seen like in our case.[6] Various target antigens have been implicated in the pathogenesis of MN, such as PLA2R, THSD7A, NELL1, semaphorin-3b, exostosin-1, and exostosin 2. NELL-1 positive MN is associated with malignancy in 30% of cases.[7] In our case of NELL-1 positive MN, there was no evidence of malignancy on screening. Evaluation for secondary cause urine mercury revealed elevated despite sampling done after 4 months of withdrawing the siddha medicine (urinary mercury level could have been higher if checked during siddha medication intake). High-level mercury exposure (urinary mercury more than 100 microgram/l) requires chelation therapy.[5] Like cases in a study by Kumar *et al*.,[5] in our case, urinary mercury was elevated but below 100 microgram/l hence managed conservatively.

She developed NELL-1 positive MN after 2 months of exposure to siddha medication possibly containing mercury (as elevated mercury was found in her urine, but siddha medication used by her could not be analyzed chemically; thus, we cannot comment conclusively), and 2 months following that, she developed anti-TPO positive euthyroid lymphocytic thyroiditis. Anti-TPO-positive thyroid disease (hypothyroidism/graves' disease) has been seen to be associated with nephrotic syndrome (the most common pathology as MN).[8] However, most of the cases with anti-TPO positive had either clinical or subclinical thyroid dysfunction at the onset of nephrotic syndrome.[8] In our case, the onset of thyroid swelling was 2 months after the onset of nephrotic syndrome, and even after 2 months from the onset of nephrotic syndrome, she was euthyroid with anti-TPO positivity, which suggests against the possibility of anti-TPO-associated MN.

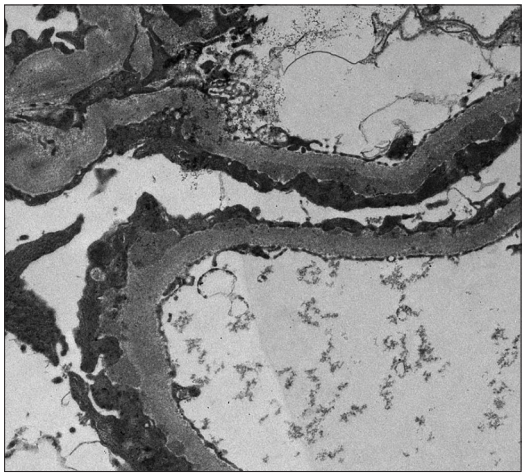

Figure 1: Electron microscopy showing subepithelial electron-dense deposits (electron microscopy ×3000). Original

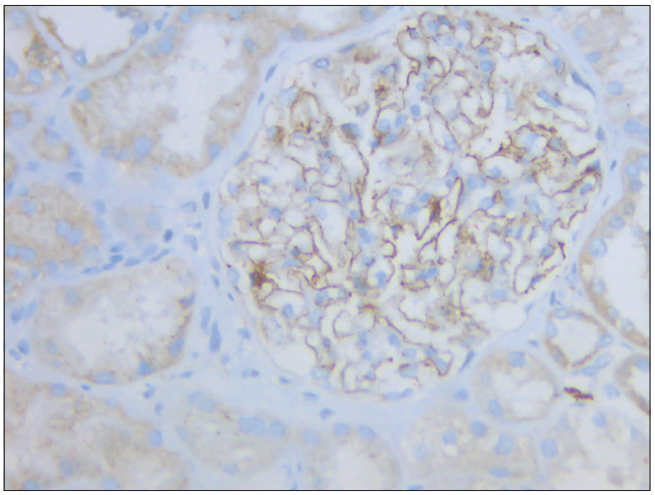

Figure 2: Immunohistochemistry showing diffuse NELL1 positivity (2+/3+) along the glomerular capillaries (NELL1 immunostain ×400). Original

Table 1: Summary of studies on mercury-related membranous nephropathy (including duration of exposure, treatment, and outcome)

Title of study	Duration of exposure (months-median with interquartile range)	Blood mercury levels (microgram/L) (Normal- < 10 microgram/l)	Urinary mercury levels (Microgram/L) (Normal-< 10 microgram/l)	Treatment offered	Outcome and Prognosis	Author of the study.
Mercury associated glomerulonephritis: a retrospective study of 35 cases in single Chinese center.[2]	5.0 (1.0, 120)	23.8 (8.2, 180.0)	29.6 (10.0,138.2)	Dimercaptosulphonate (DMPS) in 79% (27/34) cases (16 cases received detoxification, 11 patients received immunosuppression followed by detoxification	Only detoxification group -14/16 (87%) patients went into complete remission over median time of 4.5 months (range 0.3, 23.0), 2 achieved complete remission after addition of immunosuppression. Immunosuppression plus detoxification group-10/11 (90.9%) attained complete remission over median time of 2.5 months (range 0.25, 23)	Qin bo-Ai *et al*.[2]
Membranous nephropathy associated with indigenous Indian medications containing heavy metals.[5]	2.5 (2,7)	27.2 (19.1, 63.1)	30.2 (19.8, 63.1)	None of the patients receive detoxification. 6/8 (75%) cases managed conservatively-4/6 (67%) went into complete remission, 1/6 (17%) attained partial remission, 1/6 (17%) had not attained remission. Two patients received steroid therapy for 6 weeks.	Patients who attained complete remission with conservative management, did it in median time 16 months (range 10.5, 17.5). One patient attained partial remission within 6 months. 2 patients received steroid therapy and both attained remission in 1 month.	Kumar *et al*.[5]
Membranous Nephropathy due to chronic mercury poisoning from traditional Indian medicine: report of five cases.	5 (2, 66)	NA	82.7 (42.4, 140.5)	2 patients received DMPS, one received tacrolimus, one managed conservatively, one lost to follow up.	2 patients who received DMPS proteinuria reduced (not fulfilling the criterion of partial remission), 1 patient who received tacrolimus attained partial remission. one patient managed conservatively, proteinuria reduced (not fulfilling the criterion of partial remission).	Doshi M *et al*.[4]

The temporal relation of indigenous medicine intake (probably containing mercury) with NELL-1 positive MN suggests NELL-1 as a possible target antigen for mercury-related MN, but the possibility of mere coincidence cannot be ruled out and requires further studies to confirm it.

In autoimmune hypothyroidism, the site of production of antibodies such as anti-TPO antibodies is the thyroid gland, removal of which reduces the concentration of autoantibodies.[9] In patients with mercury exposure, mercury accumulation in the thyroid gland is seen.[10] Probably in our case, it is possible that mercury accumulation in the thyroid gland might have stimulated T-cell-mediated B-cell activation, leading to autoantibody formation (e.g., anti-TPO antibody).

Neural epidermal like growth factor like 1 (NELL-1) is a 90-kDa protein kinase C binding protein containing a secretory peptide predominantly expressed in osteoblasts

and renal tubules. It is hardly expressed in the glomerulus, but 5%–25% glomerular cells express NELL-1 at the mRNA level.[11] As NELL-1 is expressed predominantly in renal tubules and mercury tends to accumulate predominantly in the proximal tubule, it is possible that renal tubulointerstitium might be the site of anti-NELL1 autoantibody.[11,12] Unlike in our case, tubulointerstitial inflammation is seen in mercury-related MN, which supports the possibility of the above hypothesis, but no data on intrarenal autoantibody production in mercury-related MN (human data or animal data) is available to the best of our knowledge.[5] As NELL-1 is predominantly expressed in renal tubules, it is possible that immune complex might have deposited or sequential antigen and antibody deposited rather than *in situ* immune complex formation in subepithelial space.[13]

Though there is no human data on the exact pathophysiology of mercury-related MN, animal studies have shown T-cell-dependent polyclonal B-cell activation-related autoantibodies as the mechanism behind mercury-related MN.[3] But no target antigen has been found for mercury-related MN. A possible mechanism behind mercury-related triggering of the autoimmune phenomenon is through complex formation with amine, carboxyl, hydroxyl, and especially thiol groups in various immune cells. By forming complexes, it causes T and B-cell activation, inhibition of Fas-mediated apoptosis of autoreactive T-cells (loss of peripheral tolerance), promotes neutrophil extracellular trap (NET) formation.[14,15] Like most of the cases in the study by Kumar *et al*.,[5] patients went into remission on discontinuation of the drug and antiproteinuric therapy; the patient in our study also had reduction in proteinuria and elevation of serum albumin on stopping siddha medication and antiproteinuric therapy.

Immunoglobulin deposits in immunofluorescence, subepithelial electron-dense deposits in electron microscopy and NELL-1 as target antigen in mercury-related MN support autoimmune pathophysiology of the disease and probably because of the similar reason, there was steroid responsiveness in a few cases in the study by Kumar *et al*.[5]

Conclusion

NELL-1 can be the target antigen for mercury-related membranous nephropathy in humans. It can be present with concomitant anti-TPO-positive lymphocytic thyroiditis due to mercury exposure. Both renal and thyroid involvement supports autoimmune pathophysiology.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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